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Research Note

Synergism exists between ethylene and methyl jasmonate in artificial light-induced pigment enhancement of 'Fuji' apple fruit peel

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Abstract

The pigment content of detached 'Fuji' apple peel was characterized in fruit exposed to ethylene and/or treated with methyl jasmonate (MJ), then irradiated with ultraviolet (UV)/white light. Peel pigments were analyzed using reversed-phase high-performance liquid chromatography coupled with scanning UV–vis absorbance detection. Treatment with MJ alone enhanced anthocyanin content, including idaein, the major anthocyanin in apple fruit. Anthocyanin content was further enhanced by treatment with MJ + ethylene. Treatment with the ethylene action inhibitor 1-MCP plus MJ reduced red coloration compared with MJ alone. Treatment with ethylene or 1-MCP alone, or ethylene + 1-MCP had no effect on anthocyanin accumulation. Production of hyperin, the major quercetin glycoside in peel tissue, was enhanced by MJ and inhibition of ethylene action with 1-MCP enhanced the impact of MJ. 1-MCP with or without MJ increased phloridzin content. Chlorogenic acid synthesis was enhanced following treatment with MJ and/or ethylene, however, treatment with 1-MCP alone or 1-MCP plus MJ resulted in reduced chlorogenic acid content. β-Carotene synthesis increased following MJ plus ethylene, but was not enhanced by MJ or ethylene alone. The results indicate synergistic or additive responses between ethylene and MJ exists for regulation of apple peel pigment synthesis pathways.
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Keywords: *Malus sylvestris* var. *domestica*; Color; Chlorophyll; Carotenoid; Light stress; 1-Methylcyclopropene; Ethylene; Methyl jasmonate

1. Introduction

Methyl jasmonate [methyl 3-oxo-2-(2-pentenyl) cyclopentane-1-acetate] (MJ) has regulatory activity in many plant biosynthetic pathways (Creelman and Mullet, 1997) including anthocyanin synthesis (Franceschi and Grimes, 1991; Feys et al., 1994; Saniewski et al., 1998a,b). A mixture of artificial UV–white light promotes anthocyanin accumulation and, consequently, red coloration of apple peel (Arakawa et al., 1985). In conjunction with ultraviolet–white light, exposure of apple fruit disks (Kondo et al., 2001) and whole apple fruit to MJ (Rudell et al., 2002) leads to increases in peel anthocyanin content. Production of other peel components including hyperin, chlorogenic acid, phloridzin, and β-carotene also increases following MJ treatment and UV–white light exposure, while (−) epicatechin and lutein are unaffected by light or MJ indicating differential modulation by MJ within these pathways (Rudell et al., 2002). MJ also promotes degreening of apple peel in the absence of light (Fan and Mattheis, 1999), and MJ application to preclimacteric fruit can promote increases in ethylene synthesis as well as increase ethylene sensitivity (Fan et al., 1997).

Anthocyanin synthesis in apple fruit can also be enhanced by exogenous ethylene or the ethylene-releasing compound, ethephon (2-chloroethyl phosphonic acid) (Blanpied et al., 1975; Faragher and Brohier, 1984; Gómez-Cordovés et al., 1996). Conversely, application of aminoethoxyvinylglycine (AVG), an ethylene synthesis inhibitor, reduces anthocyanin accumulation (Wang and Dilley, 2001). While inhibition of ethylene action by 1-MCP is known to slow degreening of 'Golden Delicious' apple peel (Fan and Mattheis, 1999), the effects of the ethylene action inhibitor 1-methylcyclopropene (1-MCP) in concert with light on anthocyanin synthesis have not been previously investigated.

The present studies were conducted to evaluate the roles of UV/white light, ethylene, and MJ in pigment metabolism of 'Fuji' apple fruit peel.

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2. Materials and methods

2.1. Fruit source

Preclimacteric 'Fuji' apple fruit were harvested randomly from several trees at 135 days after full bloom at Columbia View Experimental Orchard near Orondo, WA, USA. Fruit (18 fruit per treatment) were divided into three statistical blocks per treatment. Fruit were exposed to 1-MCP the day of harvest, and other treatments were applied beginning the day after harvest.

2.2. Peel color analysis

Following harvest, the peel of each apple was marked at two sites on the shaded side (not facing the sun on the tree or non-colored side) and color at each site determined using a Minolta CR-200 colorimeter (Minolta Corp., Osaka, Japan). Peel color was measured on all 18 fruit per treatment. Measurements were obtained using the CIE L* (light to dark), a^* (green to red), b^* (blue to yellow) color space, then a^*b^* values were converted to hue angle (h°, $\tan^{-1} b/a$; McGuire, 1992). Initial h° values were subtracted from final h° values and represented as change in hue angle (Δh°) (Fig. 1). Color measurements were performed prior to and following light treatments.

2.3. Chemicals

Ethephon (21.7%, w/v; Ethrel® formulation) was obtained from Rhône-Poulenc Ag Company (Research Triangle Park, NC, USA). 1-MCP (0.14%, w/v; EthylBloc® formulation) was obtained from Floralife®, Inc. (Walterboro, SC, USA). MJ and Tween® 20 (polyethylenesorbitan monolaurate) were obtained from Aldrich (Milwaukee, WI, USA).

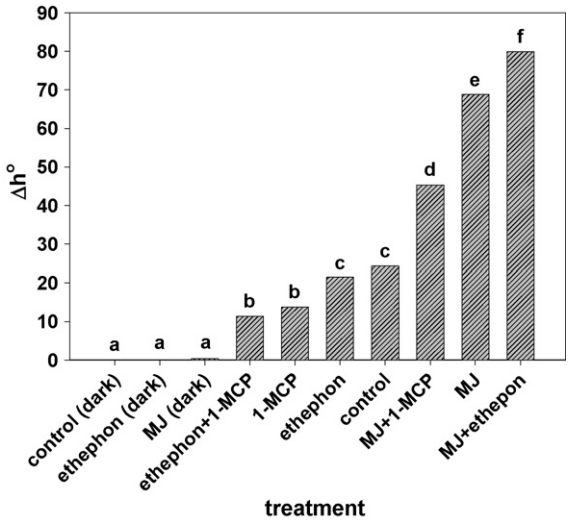

Fig. 1. Change in 'Fuji' apple hue angle ($h_{initial}{}^\circ - h_{final}{}^\circ = \Delta h^\circ$) induced by postharvest UV–white light irradiation. Apples were exposed to 0 or 10 μL L^{-1} 1-methylcyclopropene (1-MCP) for 16 h, then immersed for 2 min in deionized water containing 0.177% Tween® 20 with 0 or 1.12 g L^{-1} methyl jasmonate (MJ), or 0 or 400 μL L^{-1} ethephon, then irradiated with UV–white light for 40 h at 25 °C. Means followed by different letters denote significant differences as determined using ANOVA and Fisher's least significant difference test ($p \leq 0.05$; $n = 18$).

2.4. Chemical treatment

1-MCP (10 μL L^{-1}) treatments were carried out as detailed by Fan and Mattheis (1999). Following 1-MCP treatment, apples were immersed for 2 min in deionized water containing 0.177% (v/v) Tween® 20 alone or with 400 μL L^{-1} ethephon. Emulsified MJ was applied with the same concentration of Tween 20 at a rate of 1.12 g L^{-1} or in combination with 400 μL L^{-1} ethephon. After treatment, apples were placed on pressed paper trays and air-dried for 15 min at 21 °C.

2.5. Light treatment

Fruit were placed approximately 15 cm (apple surface to lights) under two 2-outlet 1.22 m (length) fluorescent light banks, each containing one 40 W Sylvania (Versailles, KY, USA) Cool White Deluxe fluorescent bulb, and one 40 W Phillips (Somerset, NJ, USA) fluorescent UV lamp. The irradiated area was delimited with aluminum foil. The marked side of each apple faced towards the light source. Light intensity was measured at various points within the chamber using a PMA2100 light meter (Solar Light Co., Philadelphia, PA, USA) for UV-A and UV-B irradiance, and a LI-250 light meter (LI-COR, Inc., Lincoln, NB, USA) for visible light irradiance (400–700 nm) to determine and avoid any variations in intensity. Light treatment chamber temperature was 25 °C, and apples were irradiated for 40 h.

2.6. Peel analysis

Prior to chemical and light treatment and immediately following exposure to artificial light, peel on the exposed sides of fruit was removed with a fruit peeler, flash frozen in liquid N_2, and then stored under N_2 gas at −80 °C. Metabolite profiling was performed on three biologically significant replications containing composite samples of peel from 6 fruit (18 fruit per treatment).

2.7. Anthocyanin/flavonoid analysis

Frozen, crushed peel tissue (0.5 g) was extracted in 2 mL 74:25:1 methanol/tetrahydrofuran/HCl (v/v) for 5 h in a covered ultra-sonic bath filled with ice-water. The bath was sonicated for the first and last hour of the extraction period. The extracts were partitioned with 3 mL hexanes. The hexanes phase was discarded and the remaining fraction was centrifuged and filtered to 0.45 μm particle size prior to analysis.

Pigment composition of the filtered extract was analyzed using reversed-phase high-performance liquid chromatography (HPLC). Samples (20 μL) were injected into a HP Series 1100 HPLC system (Hewlett Packard, Palo Alto, CA, USA) equipped with a 5 μm HP Hypersil ODS (4.0 mm × 125 mm) column and a Waters 996 photo-diode array (PDA) detector (Waters, Millford, MA, USA). Elution solvents were: (A)

1:10:89 H_3PO_4/methanol/deionized water (v/v) and (B) 1:70:29 H_3PO_4/methanol/deionized water (v/v). The column temperature was 25 °C and the solvent flow rate was 1 mL min^{-1}. Solvent A was used for the first 2 min, then a linear gradient of A plus B was initiated reaching 20% A and 80% B at 34 min. The rate then increased ending at 100% B after 36 min. Chromatograms from 280, 328, 357, and 519 nm wavelengths were extracted and used for quantification.

2.8. Carotenoid/chlorophyll analysis

Approximately 1 g frozen, crushed peel tissue from each sample was washed repeatedly, in the presence of 56 mg $CaCO_3$, with cold 75:25 methanol/tetrahydrofuran (v/v), until colorless. The combined washes, on ice, were partitioned with hexanes until the hexanes phase was colorless. The hexanes phase was then dried under a stream of purified N_2 gas at room temperature. Pigments were dissolved in 75:25 methanol/tetrahydrofuran (v/v) and clarified using centrifugation (10,000 × *g* for 5 min) and filtered to 0.45 μm particle size prior to analysis. Light exposure was minimized throughout the entire procedure.

Samples were analyzed immediately following extraction using the same HPLC system described previously. Solvents used for elution were: (A) 80:20 methanol/deionized water (v/v) and (B) ethyl acetate. The flow rate was 1.0 mL min^{-1}. Solvent A comprised the entire mobile phase for the first 2 min, then solvent B increased linearly reaching 50% at 21 min. This mixture was maintained until the end of the analysis at 33 min. A chromatogram from 446 nm was extracted and used for quantification.

2.9. Peak identification and quantification

Specific peaks were identified using spectral and retention comparisons with authentic standards and quantified by response comparison with authentic standards. The wavelengths at which a peak had its greatest response and least interference were used for quantification. Chlorogenic acid, phloridzin, and β-carotene were purchased from Sigma (St. Louis, MO, USA). Hyperin and idaein were purchased from Indofine (Somerville, NJ, USA).

2.10. Statistical design and analyses

Experiments were conducted using a randomized complete block design with 11 treatments with 18 fruit harvested per treatment divided into three statistical blocks each containing 6 fruit. Analysis of variance and Fisher's least significant difference tests were performed using SAS (SAS Inst., Inc., Cary, NC, USA).

3. Results

Treatment with MJ alone followed by UV–white light irradiation resulted in enhanced red coloration and anthocyanin (idaein) production (Fig. 1; Table 1). MJ + ethephon enhanced and MJ + 1-MCP reduced Δh° and idaein production when compared to MJ alone. Nevertheless, Δh° and idaein production by 1-MCP + MJ-treated fruit was higher than control fruit. Ethephon treatment alone did not impact hue angle, however, 1-MCP and ethephon + 1-MCP treatments reduced Δh°. Idaein content remained unchanged following treatment with ethephon or 1-MCP, individually or in combination. No changes in hue angle (Fig. 1) or pigment constituents (not reported) were observed in fruit held in the dark following chemical treatments.

Hyperin, a quercetin glycoside in apple peel that accumulates more compared to similar compounds following MJ plus UV–white light treatment, also was enhanced following MJ treatment (Table 1). However, unlike idaein, 1-MCP + MJ enhanced hyperin content, while hyperin content in control, 1-MCP-treated, and MJ + ethephon-treated fruit were similar. Similarly, peel phloridzin content was unaffected by ethylene released by ethephon treatment or inhibition of ethylene action by 1-MCP. All MJ treatments stimulated phloridzin accumulation, and similar to hyperin, phloridzin concentrations were highest in peel of fruit treated with 1-MCP + MJ prior to irradiation. Likewise, peel phloridzin content of 1-MCP-treated fruit was similar to that of fruit treated with MJ or MJ + ethephon.

Chlorogenic acid content was enhanced by MJ and ethephon, enhanced further by MJ + ethephon, and reduced by 1-MCP relative to irradiated controls. This pattern contrasts with that of

Table 1
Pigment content in 'Fuji' apple peel

	Idaein (mg kg^{-1})	Hyperin (mg kg^{-1})	Phloridzin (mg kg^{-1})	Chlorogenic acid (mg kg^{-1})	β-Carotene (mg kg^{-1})
Initial	1.70 a*	219 a	117a	129 a	1.37 a
Control	37.4 b	936 bcd	390 be	393 c	2.15 cd
Ethephon	23.8 ab	722 b	445 c	471 d	1.65 ab
1-MCP	28.9 b	905 bc	507 cd	254 b	1.85 bc
Ethephon + 1-MCP	22.1 ab	726 b	450 c	358 c	2.16 cd
MJ	132 d	1270 d	658 de	493 d	2.57 d
MJ + 1-MCP	100 c	1370 e	785 e	386 c	2.25 cd
MJ + ethephon	262 e	1140 cd	636 de	669 e	3.34 e
LSD	24.3	349	152	59.8	0.455

Apples were exposed to 0 or 10 μL L^{-1} 1-methylcyclopropene (1-MCP) for 16 h, then immersed for 2 min in deionized water containing 0.177% Tween® 20 with 0 or 1.12 g L^{-1} methyl jasmonate (MJ), or 0 or 400 μL L^{-1} ethephon, then irradiated with UV–white light for 40 h at 25 °C.

* Means followed by different letters in column denote significant differences as determined by ANOVA and Fisher's least significant difference test ($p < 0.05$; $n = 3$).

other peel components analyzed and indicates chlorogenic acid metabolism may be regulated by both MJ and ethylene. Synergism between MJ and ethylene was also apparent for β-carotene production where only treatment with MJ + ethephon lead to significantly higher production compared to other treatments.

4. Discussion

Altered pigment metabolism induced by MJ in concert with ultraviolet–visible light has previously been observed in apple peel (Rudell et al., 2002). The current study corroborates those results and also demonstrates synergism may exist between MJ and ethylene for regulation of multiple synthesis pathways in apple peel.

Unlike the current study, previous evidence generally points to the involvement of ethylene alone in promoting anthocyanin production in apples (Blanpied et al., 1975; Gómez-Cordovés et al., 1996; Awad and de Jager, 2002), possibly by increasing enzymatic activity in the phenylpropanoid pathway (Faragher and Brohier, 1984; Li et al., 2002). The current results indicate that ethylene or 1-MCP alone had little effect on anthocyanin production, a similar finding to that reported with peel degreening of 'Fuji' apples by Fan and Mattheis (1999). Discrepancies between this and other studies are likely due to variations in experiment length, light environment, fruit maturity or cultivar differences.

Ethylene and MJ acted synergistically or additively, with ethylene enhancing and 1-MCP reducing anthocyanin enhancement by MJ while ethylene had little consequence alone. Kondo et al. (2001) found that treatment with AVG only had a limited effect on anthocyanin accumulation when coupled with MJ treatment suggesting MJ can promote anthocyanin formation in the absence of ethylene.

Chlorogenic acid synthesis was one process that showed strong regulation by both MJ and ethylene. Previous work demonstrated that field ethephon application did not lead to increases in chlorogenic acid (Awad and de Jager, 2002) and the exposed and unexposed sides of 'Elstar' apple peel contained equal amounts of chlorogenic acid (Awad et al., 2000). 'Delicious' apples treated at harvest with 1-MCP had less chlorogenic acid biosynthesis during cold storage compared with untreated controls (MacLean et al., 2006). Again, differences between studies may be a result of the various experimental conditions used in these studies. MJ promotion of hyperin and phloridzin synthesis also seemed to be associated with ethylene activity as inhibition of ethylene perception by 1-MCP enhanced hyperin and phloridzin accumulation.

β-Carotene content increased with irradiation and MJ + ethephon. Previous reports demonstrated that β-carotene production increases with MJ treatment in dark conditions (Perez et al., 1993) and at a more rapid rate with MJ plus artificial irradiance (Rudell et al., 2002). β-Carotene content was not enhanced by MJ in the dark within the time-frame of this study. This result may indicate that, similar to the phenylpropanoid-derived peel constituents, the isoprenyl-derived β-carotene may also be enhanced by an interaction between MJ and ethylene.

Results in this and previous studies appear to indicate that differential regulation by MJ and ethylene, alone or collectively, occurs within the phenylpropanoid pathway. MJ and ethylene are also considered to function alone or collectively to mediate wound responses (McDowell and Dangl, 2000; Kunkel and Brooks, 2002) including up-regulation of portions of the phenylpropanoid pathway (Creelman and Mullet, 1997), a pathway often associated with stress responses (Dixon and Paiva, 1995). Cooperative gene regulation has been demonstrated by these plant growth regulators (Kunkel and Brooks, 2002) and the synergism or additive enhancement of peel components reported in this study may be another instance of this phenomenon. Responses in the current study were all dependent on fruit exposure to UV–vis irradiation, a finding consistent with previous reports. Light and low temperature enhanced levels of many genes associated with phenylpropanoid metabolism, including anthocyanin synthase (Ubi et al., 2006). Interestingly, (−) epicatechin and condensed tannin synthesis, peel constituents that are unaffected by ethylene (Awad and de Jager, 2002) or MJ (Rudell et al., 2002), seem to be regulated separately from other phenylpropanoid biosynthesis genes in apple peel (Takos et al., 2006).

Enhancement of these peel components, all of which have photoprotective (Merzlyak and Chivkunova, 2000) and antioxidative (Rice-Evans et al., 1996) properties, may improve product marketability by augmenting pigmentation and nutrition of apple fruit. While MJ can enhance synthesis of many of these components alone, ethylene provides additional effectiveness to the MJ treatment. Conversely, preharvest applications of ethylene synthesis or action inhibitors that reduce peel red coloration may be overcome by coincident MJ treatment.

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